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A PCR protocol for the identification of *Pseudomonas syringae* pv. *tagetis* based on genes required for tagetitoxin production

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Abstract

A polymerase chain reaction (PCR) protocol that can be used to distinguish *Pseudomonas syringae* pv. *tagetis* from other *P. syringae* pathovars, including those that induce apical chlorosis in several plants of the Asteracaea family and in pea, and closely related *P. savastanoi* pathovars was developed based on DNA sequences from *P. syringae* pv. *tagetis* that are required for tagetitoxin synthesis. PCR primer sets designated TAGTOX-9 and TAGTOX-10 in PCR amplifications with DNA from most strains of *P. syringae* pv. *tagetis*, produced amplicons of 507 and 733 bp, respectively. The same size amplicons were produced in PCR amplifications with bacterial cells isolated from chlorotic leaf tissue from Canada thistle (*Cirsium arvense*) plants infected with *P. syringae* pv. *tagetis*. Among 16 other *P. syringae* pathovars, only PCR amplifications with DNA from *P. syringae* pv. *helianthi* produced the same size amplicons with the respective primer sets. Low levels of the 507-bp amplicon were produced in PCR amplifications with the TAGTOX-9 primers and DNA from *P. syringae* pv. *helianthi* or the nontoxigenic strains of *P. syringae* pv. *tagetis*. These results suggest that *P. syringae* pv. *helianthi*, the most closely related pathovar to *P. syringae* pv. *tagetis*, may be a nontoxigenic form of *P. syringae* pv. *tagetis*. Results from PCR amplifications with the TAGTOX-9 and TAGTOX-10 primers provide strong evidence that the newly described *Pseudomonas syringae* pathovars, CT99B016C isolated from Canada thistle and PP105 and Pisum97-1 isolated from pea, which cause apical chlorosis in these respective hosts, are different from *P. syringae* pv. *tagetis*.
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Keywords: Tagetitoxin; *Pseudomonas syringae* pv. *tagetis*; *Pseudomonas syringae* pv. *helianthi*; *Pseudomonas syringae* pv. *pisi*; Apical chlorosis; *Cirsium arvense*; Canada thistle; *Helianthus annuus*; Sunflower; *Pisum sativum*; Pea; Polymerase chain reaction

1. Introduction

Pseudomonas syringae pv. *tagetis* was first described by Hellmers (1955) as a disease of African marigold (*Tagetes erecta* L.). Since that initial report, it has been isolated from French marigold (*T. patula* L.), sunflower (*Helianthus annuus* L.), Jerusalem artichoke (*Ambrosia tuberosa* L.), common dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers), common ragweed (*A. artemisiifolia* L.), giant ragweed (*A. trifida* L.), Canada thistle (*Cirsium arvense* (L.) Scop.), compass plant (*Silphium perfoliatum* L.), and willowleaf sunflower (*H. salicifolius* A. Dietr.) (Gulya et al., 1981; Johnson and Wise, 1991; Rhodehamel and Durbin, 1985; Rhodehamel and Durbin, 1989a; Shane and Baumer, 1984; Styer and Durbin, 1982; Trimboli et al., 1978). The bacterium is widely distributed, having been isolated from plants in several locations in the United States, as well as in Canada, Australia, Mexico, South America, and Czechoslovakia (Atlas de Gotuzzo et al., 1988; Cåton and Zak, 1991; Gulya et al., 1981; Johnson and Wise, 1991; Kůdela and Zacha, 1998; Laberge and Sackston, 1986; Rhodehamel and Durbin, 1985; Shane and Baumer, 1984; Trimboli et al., 1978). The most notable characteristic of the disease caused by *P. syringae* pv. *tagetis* is the apical chlorosis that develops in infected plants, the result of

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Table 1
Pseudomonas species, pathovars, and strains used in this study

Species	Host/common name or pertinent feature(s)	Country of origin	Source[a]
Pseudomonas savastanoi			
pv. *glycinea* 8727[b]	*Glycine max* L. Merr./soybean	New Zealand	ATCC
pv. *phaseolicola* 19304[b]	*Phaseolus vulgaris* L./bean	Canada	ATCC
pv. *savastanoi* 4352[b]	*Olea europaea* L./olive	Yugoslavia	ICMP
Pseudomonas syringae			
pv. *actinidiae* 9617[b]	*Actinidia deliciosa* (Chev.)/kiwi	Japan	ICMP
pv. *antirrhini* 4303[b]	*Antirrhinum majus* L./snap dragon	UK	ICMP
pv. *atrofaciens* 4394[b]	*Triticum aestivum* L./wheat	Hungary	ICMP
pv. *coronafaciens* 3113[b]	*Avena sativa* L./oat	UK	ICMP
sp. CT99B016C	*Cirsium arvense* (L.) Scop./Canada thistle	Canada	Zhang
pv. *delphinii* 529[b]	*Delphinium* sp./delphinium	New Zealand	ICMP
pv. *garcae* 19864[b]	*Coffea arabica* L./coffee	Brazil	ATCC
pv. *helianthi* 4531[b]	*Helianthus annuus* L./sunflower	Mexico	ICMP
pv. *pisi* 2452[b]	*Pisum sativum* L./pea	New Zealand	ICMP
pv. *pisi* Pisum97-1	*Pisum sativum*/pea	Japan	Takikawa
pv. *pisi* PP1	*Pisum sativum*/pea	Japan	Takikawa
pv. *pisi* PP105	*Pisum sativum*/pea	Japan	Takikawa
pv. *papulans* 4048[b]	*Malus domestica* (Borkh.) Borkh./apple	USA	ICMP
pv. *striafaciens* 3961	*Avena sativa* L./oat	Unknown	ICMP
pv. *syringae* B-1631[b]	*Syringa vulgaris* L./common lilac	UK	ARSCC
pv. *tabaci* 2835[b]	*Nicotiana tabacum* L./tobacco	Hungary	ICMP
pv. *tagetis* 1–0392	*Tagetes erecta* L./marigold	USA	Johnson
pv. *tagetis* 1–502a M2	*Cirsium arvense*/Canada thistle	USA	Johnson
pv. *tagetis* 1–1065x	*Ambrosia trifida* L./giant ragweed	USA	Johnson
pv. *tagetis* 1–1332a	*Helianthus annuus*/sunflower	USA	Johnson
pv. *tagetis* 1–1394	*Ambrosia artemisiifolia* L./common ragweed	USA	Johnson
pv. *tagetis* 1–2386	*Ambrosia artemisiifolia*/common ragweed	USA	Johnson
pv. *tagetis* 4091[b]	*Tagetes erecta*/marigold	Zimbabwe	ICMP
pv. *tagetis* 4092	*Tagetes erecta*/marigold	UK	ICMP
pv. *tagetis* 5866	*Tagetes erecta*/marigold, same as DAR26807	Australia	ICMP
pv. *tagetis* 6369	*Tagetes erecta*/marigold	Australia	ICMP
pv. *tagetis* 6371	*Tagetes* sp./marigold	USA	ICMP
pv. *tagetis* 6564	*Tagetes erecta*/marigold	USA	ICMP
pv. *tagetis* 26808	*Tagetes erecta*/marigold	Australia	DAR
pv. *tagetis* 26816	*Tagetes erecta*/marigold	Australia	DAR
pv. *tagetis* 43127	*Taraxacum officinale*/common dandelion	USA	ATCC
pv. *tagetis* 43128	*Ambrosia artemisiifolia*/common ragweed	USA	ATCC
pv. *tagetis* 349392	*Cynara scolymus* L./globe artichoke	New Zealand	CABI
pv. *tagetis* 349393	*Helianthus tuberosus* L./Jerusalem artichoke	New Zealand	CABI
pv. *tagetis* APC5	*Helianthus annuus*/sunflower	South Africa	Fourie
pv. *tagetis* APC10	*Helianthus annuus*/sunflower	South Africa	Fourie
pv. *tagetis* APC17	*Helianthus annuus*/sunflower	South Africa	Fourie
pv. *tagetis* APC18	*Helianthus annuus*/sunflower	South Africa	Fourie
pv. *tagetis* APC20	*Helianthus annuus*/sunflower	South Africa	Fourie
pv. *tagetis* APC21	*Helianthus annuus*/sunflower	South Africa	Fourie
pv. *tagetis* APC22	*Helianthus annuus*/sunflower	South Africa	Fourie
pv. *tagetis* EB037	*Ambrosia artemisiifolia*/common ragweed	USA	Johnson
pv. *tagetis* M1-1	*Helianthus annuus*/sunflower	USA	Fett
pv. *tagetis* L7	*Helianthus annuus*/sunflower	USA	Fett
pv. *tagetis* Q1	*Helianthus annuus*/sunflower	USA	Fett
pv. *tomato* 2844[b]	*Lycopersicon esculentum* Mill./tomato	UK	ICMP

[a] ATCC, American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA; ICMP, International Collection of Microorganisms from Plants, Landcare Research, 120 Mt. Albert Road, Auckland 3, New Zealand; Wenming Zhang, Alberta Research Council, Vegreville, Alberta, Canada; Yuichi Takikawa, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizoka 422-8529, Japan; ARSCC, Agricultural Research Service Culture Collection, Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, Illinois 61604, USA; David R. Johnson, Encore Technologies, Calson Business Center, 111 Cheshire Lane, Suite 500, Minnetonka, MN 55305, USA; DAR, DAR, Australian Collection of Plant Pathogenic Bacteria, Agricultural Institute, Forest Rd., Orange 2880, Australia; CABI, CABI Bioscience, Genetic Resources Collection, Bakeham lane, Egham, Surrey, TW20 9TY, United Kingdom; Deidré Fourie, ARC-Grain Crops Institute, Private Bag X 1251, Potchefstroom 2520, South Africa; and William F. Fett, USDA, ARS, Food Safety Intervention Technologies Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA.

[b] Pathotype.

the disruption of chloroplast development by tagetitoxin (Lukens and Durbin, 1985; Lukens et al., 1987; Mathews and Durbin, 1990). However, not all bacterial pathogens identified as *P. syringae* pv. *tagetis* produce tagetitoxin *in planta* (Hellmers, 1955; Rhodehamel and Durbin, 1989b; Shane and Baumer, 1984; Styer et al., 1980; Trimboli et al., 1978).

Since the first isolation of *P. syringae* pv. *tagetis* from Canada thistle plants displaying apical chlorosis (Johnson and Wise, 1991), there has been an interest in developing this bacterium as a biological weed-control agent. The pathogen has been evaluated for the control of Canada thistle in corn and soybean (Gronwald et al., 2002; Hoeft et al., 2001; Johnson et al., 1996) and for woollyleaf bursage (*Ambrosia grayi* (A. Nels.) Shinners) (Sheikh et al., 2001). Extensive efficacy trials (Abbas et al., 1999) and studies on formulations to improve shelf life (Zidack and Quimby, 2002) have also been conducted.

Recently, *P. syringae* pathovars other than *P. syringae* pv. *tagetis* have been reported to cause apical chlorosis in Canada thistle (Zhang et al., 2002) and pea (*Pisum sativum* L.) (Suzuki et al., 2003). If bacterial pathovars capable of causing apical chlorosis in field crops, such as the *P. syringae* pv. *pisi* strains as described by Suzuki et al. (2003), are present in areas where *P. syringae* pv. *tagetis* is applied to control Canada thistle, the apical chlorosis in the field crop may be interpreted as a nontarget effect of *P. syringae* pv. *tagetis.* To avoid such confusion, a simple means of distinguishing *P. syringae* pv. *tagetis* from other apical chlorosis-inducing bacterial species is needed. To address this problem, we report here on the development of a PCR protocol to distinguish *P. syringae* pv. *tagetis* from other *P. syringae* pathovars and closely related species based on genes required for tagetitoxin production.

2. Material and methods

2.1. Bacterial strains and DNA isolation

The bacterial strains used in this study (Table 1) were grown at 28 °C in King's medium B (KB) (King et al., 1954) broth or solid KB medium. Total DNA from bacteria was isolated using a genomic DNA isolation kit (MBI Fermentas, Hanover, MD) and further purified using standard chloroform–phenol extraction procedures (Sambrook et al., 1989).

2.2. PCR analysis

A 21-mer oligonucleotide with the sequence 5′-CCC GCAGTGCTGGCTTACAAC (primer TAGTOX-9 FP1) and a 19-mer oligonucleotide with the sequence 5′-TGAGCAACGCGGCCATAGC (primer TAGTOX-9 RP1) were designed from the *exbD* gene of *P. syringae* pv. *tagetis* EB037 (GenBank Accession No. AY228178) to produce a 507-bp amplicon in PCR amplifications with genomic DNA from *P. syringae* pv. *tagetis*. A 19-mer with the sequence 5′-TACCCGTGAGGCA GTGGCA (primer TAGTOX-10 FP1) and a 22-mer with the sequence 5′-TTTGAACTTGCCGGGGATA CGG (primer TAGTOX-10 RP1) were designed from the *asnB* gene of *P. syringae* pv. *tagetis* EB037 (GenBank Accession No. AY228179) to produce a 733-bp amplicon in PCR amplifications with genomic DNA from *P. syringae* pv. *tagetis.*

PCR amplifications were performed in a total of 25 μl. Reaction mixtures consisted of 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 μM each primer, 200 μmol each dNTP, 1 U AmpliTaq Gold (Perkin–Elmer, Norfolk, CT), and 40 ng of bacterial DNA or 1 μl of a bacterial cell suspension. The reactions were performed in a Perkin–Elmer GeneAmp PCR System 9600. Denaturation was at 95 °C for 10 min for the first cycle and 94 °C for 30 s for each subsequent cycle. Annealing and elongation were at 50 °C for 30 s and 72 °C for 30 s, respectively, for 5 cycles adding 2 s to the annealing time per cycle. Annealing and elongation for an additional 25 cycles were at 50 and 72 °C for 30 s adding 1 s to the annealing and extension times per cycle. Reactions were terminated after a final 5-min elongation at 72 °C. The amplicons in the reaction mix were separated by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and photographed under UV illumination by using a Eastman Kodak Company DC290 digital camera (Rochester, New York). All parts of this study were done at least twice.

2.3. Bioassays for apical chlorosis and isolation of bacteria from chlorotic Canada thistle tissue

Sunflower plants (*Helianthus annuus* cv. 'Autumn Beauty') and peas (*Pisum sativum* cv. 'Little Marvel') were grown in 50-cell flats containing Jiffy Mix Plus (Jiffy Products of America, Batavia, IL) at 24 °C under 600 $\mu mol^{-2} s^{-1}$ for a 16-h photoperiod. After 7 days, plants were inoculated by stabbing them just below the cotyledons with a 25-gauge hypodermic needle carrying cells from a 2-day-old bacterial culture grown at 28 °C on King's medium B agar. Sunflower plants were evaluated for apical chlorosis 5–7 days after inoculation and pea plants were evaluated 15 days after inoculation. Bioassays were repeated at least three times for strains that proved nontoxigenic.

Canada thistle plants were grown from seed in 10-cm-diam pots containing Jiffy Mix Plus. At 4 weeks after planting, plants were stab-inoculated in the lower stem area as described above. At 8 days after inoculation, a newly developed chlorotic leaf above the point of inoculation, about 200 mg fresh weight, was harvested and surface-sterilized by soaking in 70% ethanol for 60 s, 0.6% sodium hypochlorite for 120 s, and rinsing twice with sterile water. The leaf was then homogenized in 1 ml of sterile water and the homogenate allowed to sit for 15 min. Serial dilutions of the homogenate were prepared, 100 μl of each dilution were plated on solid KB medium, and the plates incubated at 28 °C. After 3 days, single colonies were suspended in 20 μl of sterile water and the resulting cell suspension was used in PCR amplifications.

3. Results and discussion

3.1. PCR analysis of bacterial strains isolated from Canada thistle

PCR amplification with the TAGTOX-9 and TAGTOX-10 primers and DNA from the *P. syringae* pv. *tagetis* 1-502a M2 produced the predicted 507-bp and 733-bp amplicons, respectively (lanes Pst, Fig. 1). No other amplicons were produced in these reactions. Bacterial cells isolated from chlorotic Canada thistle leaves from plants inoculated with *P. syringae* pv. *tagetis* 1–502a M2 were analyzed by PCR using the TAGTOX-9 and TAGTOX-10 primers. PCR amplification with bacterial isolates 1, 2, 4, 5, and 6 produced the 507-bp and 733-bp amplicons with the respective primer sets, while isolates 3 and 7 did not (Fig. 1). It was further demonstrated that only the isolates that produced the 507-bp and 733-bp amplicons caused apical chlorosis in the sunflower bioassay (data not shown). The results of PCR amplification and sunflower bioassays of over 50 other bacterial isolates from the same tissue described above were consistent with the above results (data not shown). We conclude that the PCR protocols described here have proved to be an effective means of distinguishing *P. syringae* pv. *tagetis* from other bacteria co-isolated from chlorotic Canada thistle leaf tissue. The PCR protocol may also prove useful in monitoring the movement of *P. syringae* pv. *tagetis* through infected plants or in identifying insect vectors that may spread the disease.

3.2. PCR analysis of various P. syringae pv. tagetis strains

All but three of the 29 PCR amplifications with the TAGTOX-9 primers and DNA from *P. syringae* pv. *tagetis* strains produced the predicted amplicon of 507 bp; this included all the toxigenic strains and the non-toxigenic strains 1–1394 and 1–2386 (Fig. 2A). Of the three samples that failed to produce the 507-bp amplicon with the TAGTOX-9 primers, the PCR amplification with DNA from strain 4092 produced an amplification product of about 750 bp (Fig. 2A, lane 19) and no PCR products were produced in PCR amplifications with DNA from strains APC10 and APC17 that were isolated from sunflower plants (Fig. 2A, lanes 21 and 22).

All but three of the 29 PCR amplifications with the TAGTOX-10 primers and DNA from *P. syringae* pv. *tagetis* strains produced the predicted amplicon of 733 bp (Fig. 2B). PCR amplifications with the TAGTOX-10 primers and DNA from the *P. syringae* pv. *tagetis* strains 349392 and 349393 failed to produce amplicons (Fig. 2B, lanes 15 and 16). These two strains were the only *P. syringae* pv. *tagetis* strains in this study that originated from New Zealand. The third *P. syringae* pv. *tagetis* strain that failed to produce the predicted 733 bp product was 4092 (Fig. 2B, lane 19). PCR amplifications with the TAGTOX-10 primers and DNA from the latter strain instead produced an amplification product of about 300 bp. The same size amplification products of 750 and 300 bp were obtained with the TAGTOX-9 and TAGTOX-10 primer sets, respectively, in PCR amplifications with DNA extracted from strain 4092 obtained from a different culture collection (Collection Française des Bactéries Phytopathogènes, Institut National de la Recherche Agronomigue, data not shown). Considering the unusual size of the amplification products produced in these PCR amplifications, and the fact that this strain failed to cause apical chlorosis in the sunflower bioassay, we believe that strain 4092 has been misidentified as *P. syringae* pv. *tagetis*. However, additional genetic analysis would be necessary to confirm this conclusion.

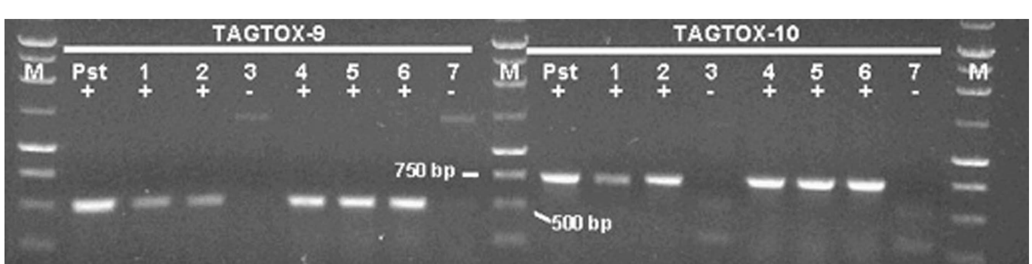

Fig. 1. Electrophoresis gel of amplicons produced by PCR amplifications with the TAGTOX-9 and TAGTOX-10 primer sets and bacterial cells isolated from chlorotic leaf tissue from Canada thistle plants inoculated with *Pseudomonas syringae* pv. *tagetis* 1–502a M2. Lanes: M, DNA marker; Pst, *P. syringae* pv. *tagetis* 1–502a M2; 1 through 7, isolates 1 through 7. Symbols + and − indicate that the strains were toxigenic or nontoxigenic, respectively, as determined by the sunflower bioassay.

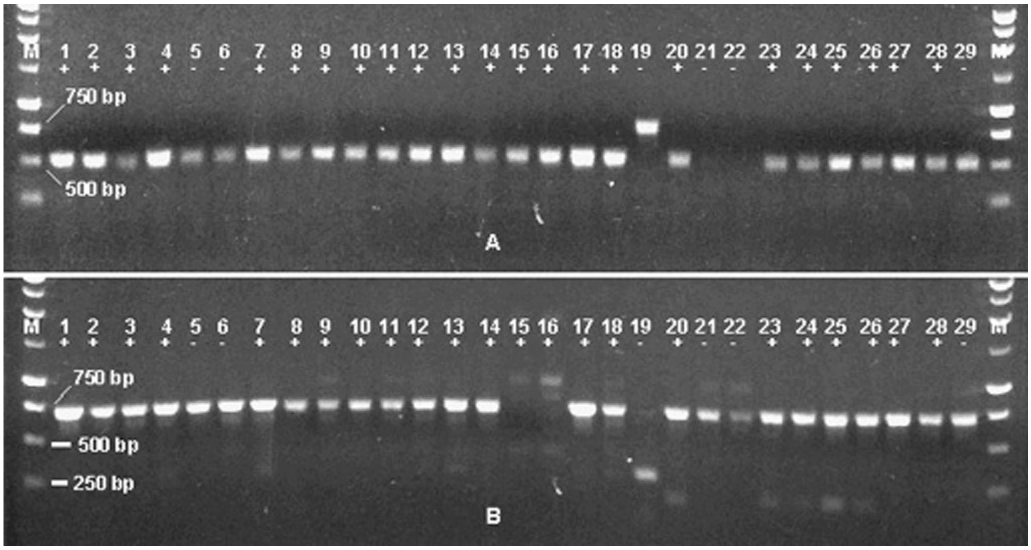

Fig. 2. Electrophoresis gels of amplicons produced by PCR amplifications with the TAGTOX-9 (A) and TAGTOX-10 (B) primer sets and genomic DNA from strains of *Pseudomonas syringae* pv. *tagetis*. Lanes: M, DNA marker; 1, 1-0392; 2, 1-502a M2; 3, 1-1065x; 4, 1-1332a; 5, 1-1394; 6, 1-2386; 7, 4091; 8, 5866; 9, 6371; 10, 6564; 11, 26808; 12, 26816; 13, 43127; 14, 43128; 15, 349392; 16, 349393; 17, EB037; 18, 6369; 19, 4092; 20, APC5; 21, APC10; 22, APC17; 23, APC18; 24, APC20; 25, APC21; 26, APC22; 27, M1-1; 28; L7; and 29, Q1. Symbols + and − indicate that the strains were toxigenic or nontoxigenic, respectively, as determined by the sunflower bioassay.

3.3. PCR analysis of various P. syringae pv. pathovars

Of the 18 pathovars tested, PCR amplifications with the TAGTOX-9 primer set and DNA from only *P. syringae* pv. *tagetis* (Fig. 3A, lanes 16 and 18), *P. syringae* pv. *helianthi* (Fig. 3A, lane 15), and *P. syringae* pv. *atrofaciens* (Fig. 3A, lane 17) produced a 507-bp amplicon. In addition, other than the PCR amplifications with DNA from *P. syringae* pv. *tagetis*, only PCR amplifications with the TAGTOX-10 primer set containing DNA from *P. syringae* pv. *helianthi* produced a 705 bp amplicon (Fig. 3B, lane 15). Excluding apical chlorosis, symptoms and biochemical and nutritional indicators are nearly identical for *P. syringae* pv. *tagetis* and *P. syringae* pv. *helianthi* (Arsenijevi et al., 1994; Fourie and Viljoen, 1994; Gulya and Banttari, 1982; Kůdela and Zacha, 1998; Laberge and Sackston, 1986; Shane and Baumer, 1984; Trimboli et al., 1978). In an

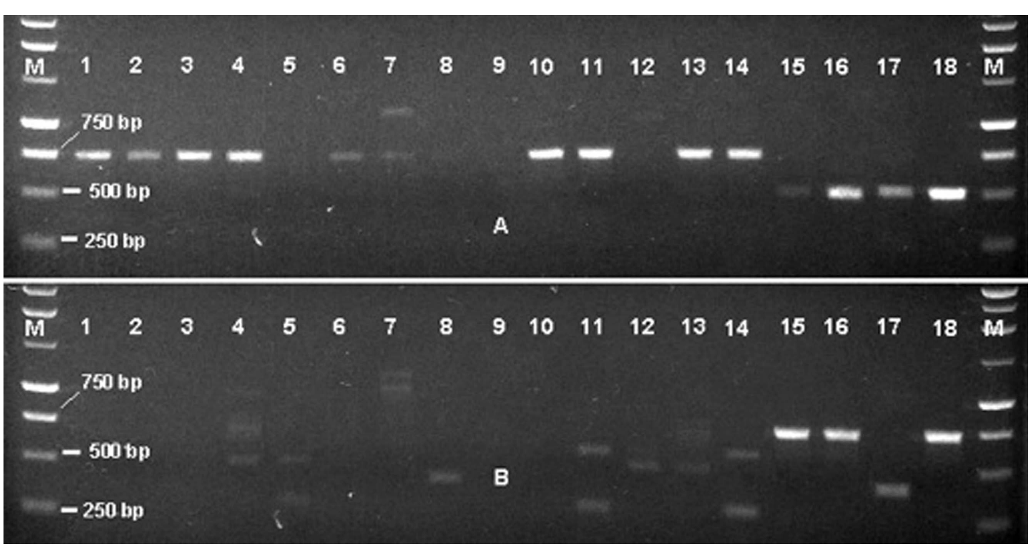

Fig. 3. Electrophoresis gels of amplicons produced by PCR amplifications with the TAGTOX-9 (A) and TAGTOX-10 (B) primer sets and genomic DNA from *Pseudomonas savatanoi* and *Pseudomonas syringae* pathovars. Lanes: M, DNA marker; 1, *P. savastanoi* pv. *glycinea* 8727; 2, *P. savastanoi* pv. *phaseolicola* 19304; 3, *P. savastanoi* pv. *savastanoi* 4352; 4, *P. syringae* pv. *coronafacines* 3113; 5, *P. syringae* pv. *delphinii* 529; 6, *P. syringae* pv. *garcae* 19864; 7, *P. syringae* pv. *pisi* 2452; 8, *P. syringae* pv. *papulans* 4048; 9, *P. syringae* pv. *syringae* B-1631; 10, *P. syringae* pv. *tabaci* 2835; 11, *P. syringae* pv. *tomato* 2844; 12, *P. syringae* pv. *actinidiae* 9617; 13, *P. syringae* pv. *striafaciens* 3961; 14, *P. syringae* pv. *antirrhini* 4303; 15, *P. syringae* pv. *helianthi* 4531; 16, *P. syringae* pv. *tagetis* 4091; 17, *P. syringae* pv. *atrofaciens* 4394; and 18, *P. syringae* pv. *tagetis* EB037.

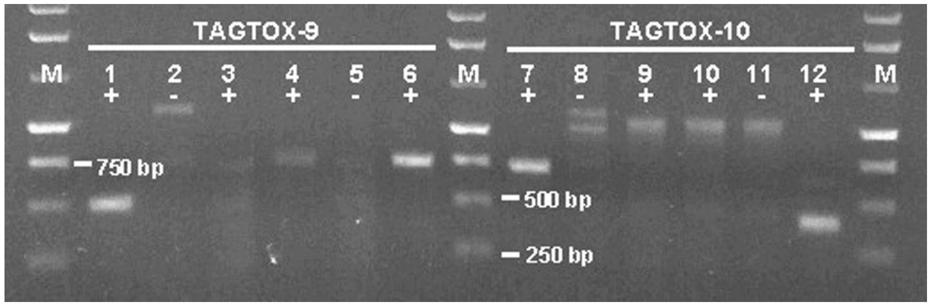

Fig. 4. Electrophoresis gel of amplicons produced by PCR amplifications with the TAGTOX-9 and TAGTOX-10 primer sets and genomic DNA from *Pseudomonas syringae* strains that cause apical chlorosis in plants. Lanes: M, DNA marker; 1 and 7, *P. syringae* pv. *tagetis* 4091 (type strain); 2 and 8, *P. syringae* pv. *pisi* 2452 (type strain); 3 and 9, *P. syringae* pv. *pisi* PP105; 4 and 10, *P. syringae* pv. *pisi* Pisum97-1; 5 and 11, *P. syringae* pv. *pisi* PP1; and 6 and 12, *P. syringae* sp. CT99B016C. Symbols + and − indicate that the strains were toxigenic or nontoxigenic, respectively, as determined by the sunflower or pea bioassay.

examination of the genetic relatedness of *P. syringae* pathovars, Gardan et al. (1999) determined that *P. syringae* pv. *helianthi* and *P. syringae* pv. *tagetis* are closely related and sufficiently distinct from other *P. syringae* pathovars, and they recommended classifying them together in a genomospecies (genomospecies 7) separate from other *P. syringae* pathovars. Our results are a further demonstration of the genetic relatedness of these two *P. syringae* pathovars. In fact, our results indicate that *P. syringae* pv. *helianthi* could possibly be a nontoxigenic form of *P. syringae* pv. *tagetis*.

3.4. PCR analysis of P. syringae pathovars causing apical chlorosis

PCR amplifications with the TAGTOX-9 and TAGTOX-10 primer sets and DNA from *P. syringae* sp. CT99B016C, a bacterial strain isolated from chlorotic Canada thistle and thought not to be a *P. syringae* pv. *tagetis* strain (Zhang et al., 2002), produced amplicons of about 750 and 400 bp, respectively (Fig. 4, lanes 6 and 12). PCR amplifications with DNA from four *P. syringae* pv. *pisi* isolates, including two described by Suzuki et al. (2003) from Japan that cause apical chlorosis in pea plants, either failed to produce amplicons or produced amplicons of a size (Fig. 4, lanes 2–5 and 8–11) different from those produced in reactions with DNA from *P. syringae* pv. *tagetis* (Fig. 4, lanes 1 and 7). This further demonstrates the utility of the TAGTOX primers in distinguishing *P. syringae* pv. *tagetis* from other *P. syringae* species, including those that cause the same symptomology in plants.

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